Efficacy of Optiphos™ phytase on mineral digestibility in diets for breeding sows: effect during pregnancy and lactation

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Abstract

Phosphorus in most diets for breeding sows is digested for 20 to 40%, thus leading to a relatively high amount of P in the manure. To enhance the P digestibility in diets for both lactating and gestating sows, two separate experiments were carried out to study the efficacy of Optiphos™ phytase derived from *E. coli* and produced by the yeast *Pichia pastoris*. Thirty crossbred gestating sows and 36 lactating sows were used in these studies. Five treatments were imposed on the gestating sows: 1) a negative control treatment, based on a low-P diet without added feed phosphate and microbial phytase. Diets in Treatments 2, 3 and 4 were the same as the negative control diet, except that an amount of Optiphos phytase of 125, 250 and 1,000 U.kg^{-1} of diet, respectively, was added. Treatment 5 was the positive control diet, based on the same diet with 1.0 g of added digestible P.kg^{-1} of diet from monocalcium phosphate. The lactating sows of Treatments 1 to 6 received a negative control diet, the same diet with an amount of Optiphos™ phytase of 125, 250, 500 and 1000 U.kg^{-1} of diet and a positive control diet supplemented with 1.5 g of digestible P.kg^{-1}, respectively. The negative control diets were different in ingredient composition because of the different nutrient requirements between lactating and gestating sows. The ratio between Ca and digestible P was kept at 2.8:1 and 3.3:1 for the lactating and gestating diets, respectively, with a minimum of 5.0 g Ca.kg^{-1}. Feeding level of the sows was according to Dutch recommendations. Six sows per treatment were used. The lactating sows received the diets from 2 weeks before farrowing until weaning of the piglets at 4 weeks of age and the gestating sows from day 49 to day 100 of pregnancy. Faecal samples of the sows were collected by rectal stimulation on days 14 and 21 post-farrowing, and on days 70 and 100 of pregnancy. Digestibility coefficients of dry matter, organic matter, ash and the minerals under investigation were calculated using Cr_2O_3 as an indigestible marker. In addition, several performance characteristics were registered. Phosphorus digestibility was clearly enhanced by the addition of microbial phytase to the lactating sow diets, as was the digestibility of ash, Ca, Na, K, Cu, and Zn. In the gestating sows only digestibility of P was significantly enhanced by microbial phytase. In both gestating and lactating sows the lowest level of phytase addition (125 U.kg^{-1} of diet) already resulted in the highest response in P digestibility without further improvement at higher phytase inclusion levels. The additional amount of digestible P absorbed with a phytase supplement of 125 or more U.kg^{-1} in lactating sows was on average 0.90 g/kg. An average amount of 0.36 and 0.67 g digestible P.kg^{-1} was generated in gestating sows at day 70 and day 100 of pregnancy by this phytase inclusion, respectively. No signs of any adverse effect of phytase on sow or piglet health and performance were observed. Optiphos™ phytase was already highly effective at a dose of 125 U.kg^{-1} of diet. Possible reasons for the lack of further improvement of P digestibility at higher doses of phytase are discussed. If feed phosphates are (partly) replaced by 125 U phytase.kg^{-1} of diet, then P excretion can be reduced by 0.85 kg.sow^{-1}.year^{-1}.

Keywords: Breeding sows, microbial phytase, minerals, digestibility, performance

1. Introduction

The present interest in microbial phytase is mainly due to the increased awareness that phosphorus from animal manure, especially pig manure, may cause environmental pollution (Poulsen et al. 1999). The majority of total phosphorus (60-80%) in plants is present in the form of phytate. Pigs only utilize phytate to a very limited extent because of the lack of phytase required for its breakdown. As a result, large amounts of unutilized phosporus are excreted with faeces. This is a major cause of environmental concern in areas with a dense livestock population. In addition, phytate can also bind with other minerals such as calcium, copper and zinc, thus reducing their availability to the animal (Lantzsch and Drochner 1988). In their reviews Dungelhoef and Rodehutscord (1995) and Jongbloed et al. (2000) showed that in most experiments on piglets and growing-finishing pigs microbial phytase improved phosphorus digestibility. The response of phosphorus digestibility to phytase depends on a number of factors, like rate of inclusion, animal category and composition of the diet. The magnitude of the response per phytase unit (U) gradually decreases in growing pigs as the level of phytase increases (Jongbloed et al. 2000) However, data on efficacy and dose-response effects of microbial phytase in breeding sows are rather scarce. Lantzsch and Drochner (1995) and Kemme et al. (1997a) reported improved P digestibility, both during gestation and lactation when using *Aspergillus niger* phytase. Also positive effects of microbial phytase in sows were reported by Jongbloed et al. (2004) and Liesegang et al. (2005). However, Kemme et al. (1997b) concluded that the efficacy of *Aspergillus niger* phytase in generating digestible P, decreased in order of lactating sows, sows at the end of pregnancy and sows at mid pregnancy. Hill et al. (2008) showed in lactating sows receiving a diet with 15% DDGS a lower P content in faeces when phytase was supplemented in their diet.

Two separate studies were performed on breeding sows, one with lactating and one with pregnant sows, with a 6-phytase preparation to assess its efficacy on the digestibility of Ca, P and some other minerals and on sow performance. Furthermore, special attention is paid to P digestibility in relation to the pregnancy and lactation stages.

2. Materials and Methods

The trials have been conducted according to the restrictions provided Animal and Human Welfare Codes/ Laboratory practice codes relevant in The Netherlands and in the spirit of 'Good Clinical Practice' as described in the VICH GL9. The experimental protocols were approved by the ethical committee of ASG, Wageningen UR, Lelystad, The Netherlands, on August 29, 2008.

The phytase preparation was derived from *E. coli* and produced by fermentation of a genetically modified yeast *Pichia pastoris* strain. It was supplied as a coated product. Huvepharma NV, Antwerp, Belgium, supplied the phytase preparation.

2.1 Gestating sows

2.1.1 Animals and housing

Thirty crossbred gestating Dutch Landrace x Yorkshire and Yorkshire x Dutch Landrace sows, were used in the trial that started on day 55 of pregnancy and ended at day 100 of pregnancy. There were three weeks between batch 1 and 2. The sows were individually housed in pens of 0.66 m wide and 2.30 m long. The first 1.80 m was a solid floor with a small slope. The remaining floor consisted of tribar metal slats, 1.5 cm thick. The feeding trough was 0.40 m wide and 0.37 m deep and was included in the length of the pen. There was space under the trough for the sow's head. The ventilation was thermostatically-controlled. Temperature has been maintained at approximately 18 °C (target).

2.1.2 Treatments and experimental design

Five treatments were imposed to the gestating sows. The first was the negative control treatment, based on a low-P by-product-based diet without feed phosphate and microbial phytase (Table 1). Diets of treatments 2, 3 and 4 were similar to the negative control diet but supplemented with 125, 250 and 1000 U phytase.kg^{-1} of diet, respectively. The fifth treatment was the positive control treatment with a diet similar to the negative control diet, with 1.0 g of digestible P from monocalcium phosphate monohydrate added. kg^{-1} of diet. Six sows per treatment were used in two periods, starting three weeks after each other, with 15 sows in each period based on their insemination date. On day 55 of pregnancy, these 15 sows were grouped in 3 blocks of 5 sows, based on their parity. Sows from each block were randomly allocated to the treatments. The sows within a block were housed as a group in adjacent pens.

Table 1. Overview of the treatments applied to the sows

Treatment/ Diet number	Treatment description	Amount added	
		Gestation diet	Lactation diet
1	Negative control	No phytase or P supplementation	No phytase or P supplementation
2	125 U	125 U.kg^{-1}	125 U.kg^{-1}
3	250 U	250 U.kg^{-1}	250 U.kg^{-1}
4	500 U	1000 U.kg^{-1}	500 U.kg^{-1}
5	1000 U	-	1000 U.kg^{-1}
6	Positive control	1.0 g digestible P.kg^{-1} as MCP	1.5 g digestible P.kg^{-1} as MCP

2.1.3 Diets and feeding of animals

Prior to commencement of the trial, the sows received a commercial gestation diet. The feed ingredients and analysed chemical composition of the experimental based diet are presented in Table 2. Major ingredients of this diet were tapioca meal, extracted sunflower seed meal, dried beet pulp, maize gluten feed and palm kernel expeller. Except for Ca and P, Dutch nutrient recommendations (CVB, 2007b) for sows were used. The content of dP in the negative control diet was estimated at 0.9 g.kg^{-1}. Based on the results of

Jongbloed et al. (2004) with Ronozyme phytase, it was assumed that 125, 250 and 1000 U of phytase kg^{-1} diet would liberate 0.35, 0.70 and 1.0 g dP.kg^{-1} of diet, respectively. Using limestone as Ca source, the Ca content of the diets was increased to obtain a constant Ca / digestible P ratio of 3.3, with a minimum of 5.0 g Ca.kg^{-1}. Further aspects on diet manufacture have been described in paragraph 2.2.3. Temperatures measured in the diets just after pelleting ranged between 67 and 72 °C. After production, the feeds were stored at room temperature until required for use. The amount of feed was offered according to an adopted schedule of the Dutch recommendations for gestating sows (CVB, 2007b). The following amounts of feed were offered: from insemination to day 42 of pregnancy 2.80 $kg.d^{-1}$, from day 43 to 81, 2.70 $kg.d^{-1}$, on days 82 and 83, 3.00 $kg.d^{-1}$ and from day 84 to farrowing, 3.25 $kg.d^{-1}$. Sows were fed twice daily, while water was available ad libitum via a nipple drinker.

Table 2. Ingredient composition, analysed chemical composition (g kg^{-1}) and nutritive value of the negative control diets

Formulation of the diets (g kg^{1})			Chemical composition and nutritive value of the diets (as fed)		
Ingredients	Lactating sows	Gestating sows		Lactating sows	Gestating sows
Barley	192.0	50.0	DM	867	876
Tapioca (starch 625-675 $g.kg^{-1}$)	200.0	232.4	Ash	60	63
Soybean meal extr. (XF<50 $g.kg^{-1}$)	130.0	52.0	XP	170	139
Peas (XP<220 $g.kg^{-1}$)	100.0	-	Crude Fat	61	50
Maize gluten feed (XP<220 $g.kg^{-1}$)	150.0	150.0	Crude Fibre	51	102
Sunflower seed extr. (XF 200-240 $g.kg^{-1}$)	40.2	100.0	Starch	261	186
Dried beet pulp (sugar 100-150 $g.kg^{-1}$)	-	170.0	Sugar	62	79
Palm kernel, expeller (XF>220 $g.kg^{-1}$)	-	80.0	NSP	253	359
Soybean hulls (XF<310 $g.kg^{-1}$)	-	51.0	Ca	5.0	5.3
Rapeseed meal extr-a.	50.0		Total P	4.5	4.0
Cane molasses (sugar<475 $g.kg^{-1}$)	65.0	50.0	Digestible P	1.3	0.9
Soybean oil	40.8	30.0	Mg	2.4	2.5
Maize starch	14.6	19.6	Na	1.9	2.0
Limestone	6.7	4.0	K	11.3	11.2
Salt	1.5	2.4	Cl	2.5	3.2
Monocalcium phosphate.$1H_2O$	0.0	0.0	NE (MJ/kg)[1]	9.14	8.80
Sodium bicarbonate	2.85	1.12	ME (MJ/kg)[1]	13.06	12.65
Vitamin and mineral mix	5.0[a]	5.0[b]	lleal dig. Lys.[1]	6.6	4.6
Chromic oxide/starch (1:3)	1.0	1.0	lleal dig. Met.[1]	2.3	1.8
L-lysine HCl	0.4	1.13	lleal dig. M+C[1]	4.4	3.1
DL-Methionine	-	0.03	lleal dig. Thr.[1]	4.5	3.3
L-threonine	-	0.42	lleal dig. Trp.[1]	1.4	0.9

[1] = from tabulated values; XP = crude protein; XF = crude fibre; NSP = non-starch polysaccharides)

[a]The vitamin-mineral premix in the lactation diet contained (per kg of diet): Vitamin A: 12000 IU; Vitamin D3: 2000 IU; Vitamin E: 40 U; Vitamin K3: 2.0 mg; Vitamin B1: 0.75 mg; Vitamin B2: 5.0 mg; d-Pantothenic acid: 15 g; Niacin; 20 mg; Biotine: 100 µg; Vitamin B12: 20 µg; Folic acid: 0.20 mg; Vitamin B6: 1.0 mg, Choline chloride: 400 mg; Fe: 150 mg; Cu: 10 mg; Zn: 65 mg; Mn: 40 mg; Co: 0.15 mg; I: 1.0 mg; Se: 0.30 mg.

[b]The vitamin-mineral premix in the gestation diet supplied per kg of diet: Vitamin A: 10000 IU; Vitamin D3: 2000 IU; Vitamin E: 25 IU; Vitamin K3: 1.00 mg; Vitamin B1: 0.75 mg; Vitamin B2: 4.0 mg; d-Pantothenic acid: 13.0 mg; Niacin: 15.0 mg; Vitamin B12: 15 µg; Folic acid: 1.3 mg; Vitamin B6: 1.0 mg, Fe: 150 mg; Cu: 20 mg; Zn: 65 mg; Mn: 30 mg; Co: 0.15 mg.

2.1.4 Collection procedures and observations

The experiment lasted 45 days, and the body weight of the sows was recorded on days 55 and 100 of pregnancy. Feed intake was recorded daily from day 55 onwards. Daily weight gain was calculated for the whole experimental period. Health status of the sows was monitored twice daily throughout the experiment. Faecal samples were taken for two days at about day 70 and day 100 of pregnancy and samples obtained on two successive days were pooled per sow.

2.2.1 Lactating sows

2.2.1 Animals and housing

Thirty-six lactating multiparous crossbred sows (Dutch Landrace x Yorkshire) and (Yorkshire x Dutch Landrace) were used in the trial. The sows received the experimental diets as soon as they were placed in a farrowing room, approx. 2 weeks before farrowing until 4 weeks after farrowing at weaning. All farrowing rooms had two rows with each six farrowing pens. Sows were individually housed in pens without bedding material. The floor of the farrowing crate was 0.65 m wide with plastic coated metal slats in the front part (1.85 m) and metal slats (0.55 m long). On every side of the sow, there was a piglet nest with a coated solid floor of 0.25 m wide and 1.20 m long. The ventilation was thermostatically-controlled and temperature was kept at approximately 22 °C. From the first week after birth, creep feed was offered to the piglets, starting with meal, followed by meal mixed with pelleted weaning feed and finally only the weaning feed. The lactating sows used in the experiment were not used for the gestating experiment in order to prevent possible carry-over effects.

2.2.2 Treatments and experimental design

The sows were subjected to six treatments. The first one was based on the negative control diet, neither supplemented by microbial phytase nor by feed phosphate (Table 1). Diets of treatments 2, 3, 4 and 5 were similar to the negative control diet, except that amounts of 125, 250, 500 and 1000 U of phytase.kg^{-1} of diet, respectively, were added. Treatment 6 was the positive control treatment

with a diet similar to the negative control diet, supplemented with 1.5 g digestible P.kg^{-1} of diet from monocalcium phosphate monohydrate, which was exchanged for maize starch.

Six sows per treatment were used in two periods, starting three weeks after each other, with 18 sows in each period based on their farrowing date. Two weeks before farrowing (day 0) the 18 sows were grouped in blocks of 6 sows based on their parity and within block randomly allocated to the dietary treatments. Litter size was standardized within two days after farrowing between 12 and 14 piglets.

2.2.3 Diets and feeding of animals

Prior to commencement of the trial, the sows had been given a commercial gestation diet. When the sows were moved to the farrowing house, they immediately received the experimental diets, without any adaptation period, until the end of the experiment. Until 4 days before farrowing 3.4 kg .d^{-1} , was offered and then until farrowing 2.5 kg .d^{-1}. On the day of farrowing 1.0 kg diet was offered and feed allowance was gradually increased to 7.0 kg at day 14 post farrowing. If more than 11 piglets were suckled 7.5 kg was offered, while 6.5 kg was offered when there were less than 11 piglets suckled.

The feed ingredients and analysed chemical composition of the basal diet are presented in Table 2. Major ingredients of this diet were barley, tapioca meal, extracted soyabean meal, peas and maize gluten feed. Before preparing the diets, the batch of barley was steam-pelleted at 82 °C to inactivate its intrinsic phytase activity. Except for Ca and P, the Dutch nutrient recommendations, as formulated by CVB (2007b) for sows were used. The content of digestible P (dP) of the negative control diet was estimated to be 1.3 g.kg^{-1}. It was assumed that Optiphos^{PM} generated 0.35, 0.75, 1.00 and 1.50 g digestible P.kg^{-1}, respectively, at doses of 125, 250, 500 and 1000 U.kg^{-1} of diet. No effect of Optiphos^{TM} on the digestibility of Ca was taken into account. Using limestone as Ca source, the Ca content of the diets was increased to obtain a constant Ca/ digestible P ratio of 2.8, with a minimum of 5.0 g Ca.kg^{-1}.

The experimental premix contained limestone, salt, vitamins and trace elements, Cr_2O_3 as a marker, maize starch as a basis to exchange phytase or monocalcium phosphate and limestone and free amino acids. The negative control diet contained neither monocalcium phosphate nor microbial phytase. Monocalcium phosphate monohydrate was included in the positive control premix. It was assumed that the digestibility of monocalcium phosphate monohydrate added was 83% (CVB, 2007a). The phytase preparation (Optiphos^{TM} 4000) contained an analysed phytase activity of 4200 U.g^{-1}.

The respective premixes and the respective amount of the phytase preparation were added to the basal diets. After that, the complete feed was mixed for five minutes and pelleted without steam addition at a moderate temperature (about 65 °C) to avoid possible inactivation of the microbial phytase. Temperatures measured in the diets just after pelleting ranged between 54 and 55 °C. Pellets were 5.0 mm in diameter. Prior to manufacture of the feeds and in between manufacture of all diets, the whole system was flushed with a large portion of the basal diet, in order to avoid any cross-contamination. This portion was discarded. The control diets were mixed in order of treatment number. After production, the feeds were stored at room temperature until required for use.

2.2.4. Collection procedures and observations

During the production of the experimental feeds, two samples of each diet were taken of which one was immeditately analysed, while the other was frozen at -18°C. At the end of the trial, another sample of each diet was taken at the trial site. Faecal samples were obtained by rectal stimulation of the sow, which was done early in the morning just before feeding and in the afternoon around 15 h. Faecal samples were stored at-18 °C pending analysis. Health status of the sows was monitored twice daily throughout the experiment. The experiment lasted 43 days, and the body weight was recorded on day 0 and day 43. Feed intake was recorded daily from d 0 to 43. Faecal samples were taken on days 27 and 34 (days 14 and 21 post-farrowing). The following data of the litters and piglets were recorded: the number of piglets born (alive and dead), birth weight and weaning weight per individual piglet and creep feed intake of the piglets per litter. Weak and dead animals that were removed from the litters were recorded, but their live weight gain was not taken into account for the total litter growth.

2.3. Analytical procedures and calculations

The feed samples taken at feed production were analysed for dry matter, ash, nitrogen, Ca, P, Na, K, Cu, Zn, Cr and phytase activity in duplicate. The negative control diets were also analysed for crude fat, crude fibre, starch, sugars, Cl and phytic acid in duplicate. Freeze-dried faecal samples were analysed for dry matter, ash, N, P, Ca, Na, K, Cu, Zn in single, and for Cr in duplicate. Dry matter, ash, nitrogen, crude fat and crude fibre contents were determined using AOAC procedures (1984). Starch content was determined enzymatically according to the amyloglucosidase/ hexokinase method (NEN 3574). Sugars were determined with a modified Luff-Schoorl method. The Ca, P, Mg, Na, K, Cu and Zn contents were determined using the inductively coupled plasma atomic emission spectrometry (ICP-AES). The Cl content was determined by potentiometric titration of water-diluted solid samples with the chloride specific ion electrode (Jenway Chloride Meter, Model PCLMP3). Chromium was assessed according to Williams et al. (1962). Phytic acid in the feeds was extracted and quantitatively determined by a HPLC method (Bos et al. 1991). Phytase activity in all diets was analyzed by Huvepharma NV in duplicate according to the assay outlined in Biovet JSC.SOP RDM-297. One unit of phytase activity is defined as the amount of enzyme which liberates 1µmol of inorganic phosphate per minute from 5.1 mmol of sodium phytate per litre at 37 °C at pH 5.5, under conditions of the test. Digestibility coefficients of dry matter, ash, organic matter and the minerals under investigation were calculated using Cr_2O_3 as an indigestible marker.

2.4. Statistical analysis

Each sow was considered as an experimental unit. Data were analyzed by GenStat (2008), release 11.1 package. Feed consumption of the sows and digestibility of dry matter, organic matter, ash, Ca, P, Mg, Na, K, Cu and Zn were compared between the treatment groups using analysis of variance. The following model was used:

$Y_{ij} = \mu + period_i + treatment\ block_j\ in\ period_i + treatment_k \times period_{ik} + e_{ijk}$

Where: μ = overall mean;

$period_i$ = period effect (i = 1,2);

$block_j$ = block effect (j = 1…3) within $period_i$;

$treatment_k$ = treatment effect (k =1…6 for lactating or k = 1…5 for gestating sows);

treatment x $period_{ik}$ = interaction of treatment and period;

e_{ij} = error contribution with average 0 and standard deviation σ^2.

Significant pairwise differences among treatments were tested by Student's t-test. Piglets born per litter, piglets lost per litter before and 24 h after birth, and the cause of Piglets losts were tested by the X^2 test (Chi-square).

3. Results

3.1. Chemical composition of the diets

The analysed chemical composition of the diets is presented in Table 3. As the variation in the P contents of the negative control diets and the diets with added microbial phytase was within the analytical error, we used the mean P content in all further calculations. The P content of the positive control diets were as expected. The graded levels of calcium were as expected except for pregnancy diet 2, which due to a mistake contained 4.0 g $Ca.kg^{-1}$ instead of 5.0 g $Ca.kg^{-1}$. This, however, has no negative effect on P digestibitlity. The variation in the analysed concentrations of Cu and Zn among diets was very low. Therefore, their contents in the different experimental diets were pooled as well, and the mean values were used for further calculations. The analysed activity of phytase in the diets, when corrected for the amount in the basal diets without phytase supplementation, were very close to the intended levels in the lactation diets, whereas in the gestation diet the analysed level of 1156 $U.kg^{-1}$ was slightly higher than the intended 1000 $U.kg^{-1}$.

Table 3. Analysed chemical composition (as fed) of experimental diets ($g.kg^{-1}$) and added phytase activity ($U.kg^{-1}$)[b]

Treatment	DM	Ash	OM	Ca	Mg	P	Na	K	Cu ($mgkg^{-1}$)	Zn ($mgkg^{-1}$)	Phytase Activity[b]
Lactation diets											
Neg. Contr.[a]	867	59.6	808	5.0	2.4	4.5	1.9	11.3	27	98	0
125 $U.kg^{-1}$	864	58.6	805	5.0	2.4	4.6	1.9	11.3	28	99	125
250 $U.kg^{-1}$	866	61.2	805	5.9	2.5	4.6	2.0	11.3	27	103	263
500 $U.kg^{-1}$	865	62.7	802	6.7	2.5	4.5	2.0	11.5	31	101	502
1000 $U.kg^{-1}$	868	65.9	802	8.0	2.5	4.6	2.0	11.5	30	101	1033
Pos. Contr.	867	67.6	800	7.7	2.5	6.3	2.0	11.6	32	101	0
Gestation diets											
Neg. Contr.[a]	876	63.0	813	5.3	2.5	4.0	2.0	11.2	24	109	0
125 $U.kg^{-1}$	873	59.6	814	4.0	2.5	4.0	1.9	11.1	22	107	121
250 $U.kg^{-1}$	875	63.5	811	5.0	2.5	4.0	1.9	11.2	22	106	270
1000 $U.kg^{-1}$	876	65.2	810	6.0	2.6	4.0	2.0	11.3	24	103	1156
Pos. Contr.	875	67.1	808	5.8	2.5	5.2	1.9	11.1	24	107	0

[a]Analysed phytate P content and phytase activity in the negative control lactation and gestation diets were 2.79 and 2.38 $g.kg^{-1}$ and 18 and 11 $U.kg^{-1}$, respectively.

[b] Corrected for the amount of intrinsic phytase in the negative control diets.

3.2 Gestating sows

3.2.1. Health status and animal performance

The trial was carried out without major problems. No sows were lost or required medical treatment. There were no feed refusals during the entire experiment. The average initial body weight (day 55 of pregnancy) was 251 kg and final body weight at day 100 of pregnancy (280 kg), were not significantly different among treatments. Average daily gain (634 g) and average daily feed intake (2.85 kg) of the sows were not significantly different among the treatments.

Table 4. Digestibility of the nutrients by gestating sows at day 70 of pregnancy (%)

Treatment	DM	Ash	OM	Ca	P	Mg	Na	K	Cu	Zn
Negative control	78.6^{ab}	42.3^{b}	81.4	25.8^{b}	22.3^{a}	28.2^{ab}	91.2	85.6^{ab}	26.1^{cd}	9.7
125 $U.kg^{-1}$	79.0^{ab}	42.3^{b}	81.7	25.7^{b}	32.2^{b}	27.4^{ab}	90.6	83.4^{a}	18.2^{a}	3.1
250 $U.kg^{-1}$	79.5^{b}	46.6^{c}	82.0	28.9^{b}	31.8^{b}	28.2^{ab}	91.3	87.5^{b}	21.7^{ab}	7.6
1000 $U.kg^{-1}$	79.0^{ab}	44.1^{bc}	81.8	26.3^{b}	30.0^{b}	30.8^{b}	91.1	88.5^{b}	28.7	7.9
Positive control	78.1^{a}	38.7^{a}	81.4	18.5^{a}	22.0^{a}	25.8^{a}	90.2	83.6^{a}	23.7^{bc}	5.5
SED[2]	0.55	1.63	0.53	3.01	2.24	1.74	2.06	1.51	1.95	4.27
P-value	0.20[1]	0.002	0.73	0.031	<0.001	0.11	0.98	0.009	<0.001	0.61

[1]abc Within column, values without common superscript are significantly different at P<0.05

[2]SED= Standard error of the difference of the means

Table 5: Digestibility of the nutrients by gestating sows at day 100 of pregnancy (%)

Treatment	DM	Ash	OM	Ca	P	Mg	Na	K	Cu	Zn
Negative control	77.2^{ab}	40.9^{a}	80.0^{ab}	28.6^{ab}	22.1^{a}	24.8^{a}	90.9	84.2^{bc}	25.9^{b}	-11.3
125 U.kg^{-1}	76.9^{a}	40.6^{a}	79.5^{a}	34.3^{bc}	38.3^{c}	23.7^{a}	91.8	81.4^{a}	16.3^{a}	-6.4
250 U.kg^{-1}	78.2^{b}	46.5^{b}	80.7^{b}	36.5^{c}	38.2^{c}	26.1^{ab}	90.8	86.4^{cd}	23.2^{b}	-1.9
1000 U.kg^{-1}	77.6^{ab}	48.5^{b}	79.9^{ab}	32.9^{bc}	40.0^{c}	29.4^{b}	93.2	87.1^{d}	31.8^{c}	-4.1
Positive control	77.0^{a}	41.4^{a}	80.0^{ab}	25.5^{a}	29.6^{b}	24.5^{a}	90.5	83.1^{ab}	26.0^{b}	-5.3
SED[2]	0.51	1.60	0.50	2.91	2.81	1.93	1.93	1.19	1.80	5.18
P-value	0.12[1]	<0.001	0.50	0.008	<0.001	0.059	0.62	<0.001	<0.001	0.47

[1]abc Within a column values with different superscripts are significantly different at P<0.05

[2]SED=Standard error of the difference of the means

3.2.2. Digestibility of nutrients

There were marked differences in the level of P digestibility between the estimates at days 70 and 100. Therefore, results of these two days are presented separately in Tables 4 and 5, respectively.

3.2.2.1. Digestibility at day 70 of pregnancy

Treatment had a significant effect (P<0.05) on the digestibility of ash, Ca, P, K and Cu, measured at day 70 of pregnancy (Table 4). Ash digestibility was highest for the diet with 250 U.kg^{-1} and significantly different from the control diets.

Calcium digestibility slightly increased when phytase was included in the diet, but only the positive control diet showed a significantly lower Ca digestibility than the other diets. Phosphorus digestibility was improved (P< 0.001) by microbial phytase at all levels of phytase supplementation as compared to the negative control treatment. There were no differences in P digestibility among the three supplementation levels of phytase (on average 31.3%). Magnesium, Na and Zn digestibilities were not affected by dietary treatment. Potassium digestibility was improved by phytase supplementation of the diet (P< 0.001) at inclusion levels of 250 and 1000 U.kg^{-1}. Copper digestibility was influenced by treatment but showed an irregular pattern among phytase addition levels.

3.2.2.2. Digestibility at day 100 of pregnancy

Treatment had a significant effect (P<0.05) on the digestibility of ash, Ca, P, K and Cu measured at day 100 of pregnancy (Table 5). Ash digestibility was highest for the diets supplemented with 250 and 1000 U.kg^{-1} phytase. Calcium digestibility was significantly affected by the dietary treatment (P=0.008) but not different between diets with supplemented phytase. Phosphorus digestibility was improved (P<0.001) by microbial phytase at all levels of phytase supplementation. There were no differences among dietary phytase levels with P digestibility being on average 38.8%. P digestibility of the negative control diet (22.1%) was again lowest of all treatments. Magnesium, Na and Zn digestibilities were not affected by dietary treatment. Potassium digestibility was improved by phytase supplementation of the diet and the highest response was observed when 1000 U.kg^{-1} was added to the diet. However, 125 U.kg^{-1} resulted in a lower K digestibility than the negative control diet. Copper digestibility was affected by dietary treatment, but phytase addition showed an irregular pattern; the highest response was observed at an inclusion level of 1000 U.kg^{-1}.

3.3 Lactating sows

3.3.1. Health status and animal performance

The trial with lactating sows was carried out without major problems. Three sows in the first period did not consume all the feed supplied. No sows were lost or medically treated for diet-related diseases. Average live weight of the sows at the start and at the end of the trial was 281 and 237 kg, respectively, and no treatment effects were observed (P>0.01). Also, no differences in feed intake before farrowing and during the lactation period were observed among treatments. Average daily feed intake during the whole experimental period was 4.39 kg. sow^{-1}. The number of piglets born alive per litter was not different among treatments and ranged from 12.0 to 16.1 Also, there were no differences between treatments in total birth weight per litter (22.1 kg), average growth rate per piglet (258 g.d^{-1}), and mortality of piglets per litter (1.6 piglet). Average weaning weight (8.2 kg) was not affected by treatment (P> 0.10).

3.3.2. Digestibility of nutrients

No significant differences in digestibilities of the nutrients (or treatment x period interactions) could be demonstrated between days 14 and 21 of the experiment except for Mg. Thus, the digestibilities were averaged and the results are presented in Table 6. Treatment significantly affected (P<0.005) the digestibility of all nutrients under investigation. The largest difference in digestibility of the nutrients was observed between the negative and positive control diets and the phytase-supplemented diets. With regard to DM, ash and OM digestibility the positive control diet showed the lowest digestibility. Calcium and phosphorus digestibility increased significantly with the inclusion of 125 U phytase per kg, without further increase at higher inclusion levels the average P digestibility of all phytase levels was 47.8% compared with 28.6% for the negative control diet. Magnesium digestibility improved with phytase from 10.2% for the negative control diet to on average 20.7% for the phytase-supplemented diets. Both sodium and potassium digestibility were enhanced by phytase inclusion, and the highest phytase levels of phytase resulted in the highest digestibility. This effect was almost significant for all four levels of phytase inclusion. Sodium digestibility was 9.1 percentage-units higher at 1000 U.kg^{-1} compared to the negative control diet, while for potassium this difference was 9.3 percentage-units. Copper and zinc digestibility was influenced by phytase addition, but the effect of the inclusion level was inconsistent.

Table 6. Average faecal digestibilities (%) in lactating sows on day 14 and 21 of lactation as affected by treatment[1]

Treatment	DM	Ash	OM	Ca	P	Mg	Na	K	Cu	Zn
Negative control	80.1^{bc}	43.7^{a}	82.8^{bc}	38.6^{b}	28.6^{a}	10.2^{ab}	84.4^{a}	81.5^{a}	9.7^{a}	-0.2^{a}
125 U.kg^{-1}	81.0^{c}	52.1^{b}	83.1^{c}	43.4^{b}	46.6^{c}	18.1^{b}	87.4^{b}	86.2^{c}	20.8^{c}	7.1^{c}
250 U.kg^{-1}	80.9^{c}	51.7^{b}	83.1^{c}	41.0^{b}	47.6^{c}	20.1^{b}	89.8^{bc}	88.1^{d}	15.7^{b}	10.3^{dc}
500 U.kg^{-1}	80.6^{bc}	50.2^{b}	83.0^{bc}	42.5^{b}	48.6^{c}	22.7^{b}	90.8^{cd}	88.9^{d}	26.4^{c}	8.8^{cd}
1000 U.kg^{-1}	79.7^{ab}	50.8^{b}	82.1^{ab}	38.4^{ab}	48.3^{c}	21.7^{b}	93.2^{d}	91.0^{e}	23.8^{d}	7.0^{c}
Positive control	78.9^{a}	44.1^{a}	81.9^{a}	32.9^{a}	35.8^{b}	-2.8^{a}	88.5^{bc}	89.5^{a}	22.8^{cd}	2.9^{b}
SED[2]	0.47	1.29	0.46	2.79	1.48	7.65	1.38	0.90	1.32	1.36
P-value	<0.001	<0.001	<0.005	<0.005	<0.001	<0.011	<0.001	<0.001	<0.001	<0.001

[1]abc Within a column values with different superscripts are significantly different at P<0.05

[2]SED=Standard error of the difference of the means

4. Discussion

This study shows that supplementing the microbial phytase Optiphos^{TM} derived from *E. coli* to low-P diets improves the digestibility of P and several other minerals in gestating and lactating sows. No negative consequences either to the performance of suckling piglets or lactating sows (number of piglets born alive and weaned or sow weight change) were observed by feeding this microbial phytase. Feed intake and weight variation were not significantly affected in gestating and lactating sows microbial phytase was added to the basal diet at any level of supplementation.

4.1 Phosphorus digestibility in gestating

The improved P digestibility by adding phytase to diets to of pregnant sows is agreement with other reports (eg. Lantzsch and Drochner, 1995; Kemme et al, 1997a, b; Jongbloed et al, 2004; Männer and Simon, 2006; Nyachoti et al, 2006; Swiatkiewicz and Hanczakowska 2008). It was surpising that digestibitlity of P in this experiment was similar at each level of supplementation between 125 and 1000 U.kg^{-1}. The effect of phytase on P digestibility was bigger at the end of gestation than in mid gestation, as reported in several other studies (Table 7). Because digestibility of Ca and P in the basal diet of Lantzch and Drochner (1995) was not available we assumed a digestibility of 25% and 20% respectively. Furthermore, the observation at 500 U.kg^{-1} in experiment 2 of Nyachoti et al. (2006) was ignored because of the much higher P content in that diet compared to the negative control diet.

4.1.1 Effect of pregnancy stage

From table 7 it can be concluded that stage of gestation may cause large differences in effect of phytase on P digestibility and generated dP. The amount of generated dp.kg^{-1} of diet was 0.75, 0.38, 0.07, 0.35, 0.93 and 0.31 higher around day 100 of pregnancy Compared with day 60.75of pregnancy as reported by Kemme et al (1997), Liesegang et al (2005), Jongbloed et al (2004), Nyachoti et al (2006), and this experiment, respectively. The average improvement in generated dP was 0.47±0.31 g.kg^{-1} diet compared with day 60-75. The experiment of Grela et al (2000) showed a slight improvement of phytase efficacy in week 14 of pregnancy compared to week 8. The difference in P digestibility and generated dP is most likely due to the higher requirement for P as pregnancy progresses. Therefore we estimated the amount of required digestible P and total Ca based on the live weight of the sow, the parity number (average) and the number of piglets born (average of each experiment), piglet birth weight of 1450g using the model of Jongbloed and Everts (1992) and Jongbloed et al (2003).

The results on the amount of Ca required in relation to the amount of total Ca provided by the diet, showed that in almost all experiments the supply of Ca was around the requirement for Ca. The exception was the experiment of Manner and Simon (2006), who supplied Ca at 200% of the requirement. A too high provision of the amount of Ca supplied may have a negative effect on the digestibility of P (Jongbloed, 1987; Selle et al. 2009).

4.1.2 Dose-response relationship between phytase and generated dP in pregnant sows

It can be concluded from Table 7 that in most experiments there was no positive relationship between dose of phytase and digestibility of P. Only Lantzsch and Drochner (1995) demonstrated a clear positive dose-response effect. Jongbloed et al (2004), Nyachoti et al (2006) and Swiatkiewicz and Hanczakowska (2008), however, could not demonstrate differences in digestibility of P when using the levels of phytase supplementation. Also in the current experiment no difference could be shown in the amount of generated dP between 125 and 1000 U.kg^{-1} as well. As discussed earlier this may be due to the fact that the supply of dP was higher than the requirement of dP. Unfortunately, we did not measure urinary P concentration to confirm this.

4.2. Phosphorus digestibility in lactating sows

The improved P digestibility by adding phytase to diets of lactating sows is in agreement with other reports (eg. Lantzsch and Drochner, 1995; Kemme et al. 1997 a,b; Jongbloed et al. 2004; Männer and Simon, 2006; Nyachoti et al. 2006; Hanczakowska et al. 2008). Surprisingly, the digestibility of P in our experiment was almost similar at each level of phytase supplementation (Table 8). Because digestibility of Ca and P in the basal diet of Lantzsch and Drochner (1995) was not available we assumed a digestibility of 25% and 20%, respectively.

4.2.1. Effect of stage of lactation

From table 8 it can be concluded that there were negligible differences in effect of phytase on P digestibility and generated dP depending on lactation date. It was calculated that the amount of generated dP (g.kg^{-1}) was on average 0.04 ± 0.31 higher in mid lactation than at end of lactation. We conclude that during lactation only one measurement is sufficient for a correct estimate of efficacy of phytase on digestibility of P, preferably between day 14 and day 20 lactation.

Table 7. Overview of effect of phytase enhancement of P digestibility (%) and on generated dP (g/kg) in pregnant sows

Phytase source	Added phytase, U/kg^{-1}	P, g/kg diet	P digestibility		Generated dP		Reference
			mid	end	mid	end	
Natuphos	250	4.1	-	16.1	-	0.66	Lantzsch and Drochner, 1995
Natuphos	500	4.1	-	23.4	-	0.96	Lantzsch and Drochner, 1995
Natuphos	500	4.8	6.7	15.0	-0.03	0.71	Kemme et al.1997a
Ronozyme	750	3.9	8.4	10.6	0.33	0.41	Jongbloed et al. 2004
Ronozyme	1000	3.9	8.4	10.6	0.33	0.41	Jongbloed et al. 2004
Ronozyme	750	4.2	4.0	13.0	0.17	0.55	Liesegang et al. 2005
Ronozyme	500	3.6	2.5	-	0.09	-	Männer & Simon, 2006
Ronozyme	750	3.6	3.1	-	0.11	-	Männer & Simon, 2006
Ronozyme	1000	3.6	9.5	-	0.34	-	Männer & Simon, 2006
E.coli	500	5.1	-	6.8	-	0.38[1]	Nyachoti et al. 2006;Expt1
E.coli	1000	4.9	-	2.6	-	0.13[1]	Nyachoti et al. 2006;Expt1
E.coli	1000	5.8	-	8.3	-	0.47[1]	Nyachoti et al. 2006;Expt2
AB Phytase	125	3.7	-	11.7	-	0.40	Swiatkiewicz & Hanczakowska, 2008
AB Phytase	250	3.7	-	12.3	-	0.46	Swiatkiewicz & Hanczakowska, 2008
AB Phytase	375	3.7	-	11.7	-	0.44	Swiatkiewicz & Hanczakowska, 2008
AB Phytase	10000	3.7	-	12.4	-	0.46	Swiatkiewicz & Hanczakowska, 2008
Optiphos	125	4.0	9.9	16.2	0.40	0.65	Present experiment
Optiphos	250	4.0	9.5	16.1	0.38	0.64	Present experiment
Optiphos	1000	4.0	7.7	17.9	0.31	0.72	Present experiment

[1] combined effect of two pregnancy states

Table 8 Overview of effect of phytase enhancement of P digestibility (%) and on generated dP (g/kg) in lactating sows

Phytase source	Added Phytase U/kg	P, g/kg diet	P digestibility		Generated dP		Reference
			Mid	End	Mid	End	
Natuphos	250	5.0	-	26.5	-	1.33	Lantzsch and Drochner, 1995
Natuphos	500	5.0	-	26.5	-	1.33	Lantzsch and Drochner, 1995
Natuphos	500	4.8	21.4	22.0	1.02	1.05	Kemme et al. 1997a
Natuphos	400	4.0	17.2	13.4	0.69	0.54	Kemme et al. 1997b
Ronozyme	750	5.0	17.2	13.5	0.86	0.67	Jongbloed et al. 2004
Ronozyme	1000	5.0	15.7	17.7	0.78	0.88	Jongbloed et al. 2004
Ronozyme	10000	5.0	23.9	23.0	1.19	1.15	Jongbloed et al. 2004
Ronozyme	750	4.5	8.0	5.0	0.36	0.22	Liesegang et al. 2005
Consensus	500	3.5	14.3	-	0.49	-	Männer & Simon, 2006
Consensus	1000	3.5	21.9	-	0.76	-	Männer & Simon, 2006
E.coli	500	4.4	-	6.8	-	0.28	Nyachoti et al. 2006; Expt 1
E.coli	1000	4.5	-	2.6	-	0.11	Nyachoti et al. 2006; Expt 1
E.coli	500	5.8	-	23.5	-	1.37	Nyachoti et al. 2006; Expt 2
E.coli	1000	5.4	-	8.3	-	0.35	Nyachoti et al. 2006; Expt 2
AB Phytase	125	4.1	-	9.5	-	0.39	Swiatkiewicz & Hanczakowska, 2008
AB Phytase	250	4.1	-	10.3	-	0.43	Swiatkiewicz & Hanczakowska, 2008
AB Phytase	375	4.1	-	11.9	-	0.49	Swiatkiewicz & Hanczakowska, 2008
AB Phytase	10000	4.1	-	12.7	-	0.53	Swiatkiewicz & Hanczakowska, 2008
Optiphos	125	4.6	18.2	18.8	0.82	0.85	Present experiment
Optiphos	250	4.6	18.0	20.3	0.82	0.92	Present experiment
Optiphos	500	4.6	18.5	20.7	0.84	0.94	Present experiment
Optiphos	1000	4.6	18.5	20.6	0.84	0.93	Present experiment

4.2.2. Dose-response relationship between phytase and generated dP in lactating sows

Data in Table 8 indicate that in the experiments of Jongbloed et al. (2004), Männer and Simon (2006) and Swiatkiewicz and Hanczakowska (2008) there was a positive relationship between dose of phytase and P digestibility, as would be expected. However, in the experiments of Lantzsch and Drochner (1995) and Nyachoti et al. (2006) similar or even lower P digestibilites were found when higher phytase doses were used. Also in the current experiment no differences could be shown in the amount of generated dP between 125 and 1000 U.kg^{-1}. It is, therefore, interesting to know the relationship between generated dP and doses of Optiphos^{TM} between 0 and 125 U kg^{-1} of diet in lactating sows.

To get more insight in the possible reasons of the absence of an increase in P digestibility at a higher dose of phytase, we estimated the amount of required digestible P and total Ca based on the live weight of the sow, the parity number (average) and the number of piglets weaned (average of each experiment), piglet growth of 240 g.d^{-1} using the model of Jongbloed and Everts (1992) and Jongbloed et al.

(2003). In this model it is not accounted for demineralization of bone of the breeding sow during lactation, because it is difficult to estimate.

When calculating the ratio between supplied and required amount of total Ca we got the following picture. In the phytase-supplemented diets a ratio between supplied and required total Ca varied between 33% and 88% in the experiments of Kemme et al. (1997b), Jongbloed et al. (2004), Liesegang et al. (2005), Nyachoti et al. (2006), Swiatatkiewicz and Hanczakowska (2008), Männer and Simon (2006) and the current experiment except at the dose of 1000 U.kg^{-1} in the current experiment where the ratio was 101%. The above estimations show that in most experiments the supply of dP and Ca was lower than the requirement of dP and total Ca, thus oversupply of dP and Ca cannot be the reason why no further improvement of P digestibility is obtained at higher phytase doses. It should be remarked that requirements of Ca can be better expressed as digestible Ca instead of total Ca but requirements based on digestible Ca are not available in literature. Thus the effect of phytase on enhancement of the Ca digestibility has not been taken into account.

Another explanation would be that microbial phytase already obtained its maximal capacity to hydrolyse phytic acid. This is not likely because digestibility of Mg, Na, K and Cu further increased at a higher dose of phytase. Based on the generated amount of digestible P and the phytate P concentration of 2.79 it could be estimated that on average only 32% of phytate P was utilized as dP. This is rather low, because in diets for piglets and growing-finishing pig often 40 to 50% is obtained (Jongbloed, 2010).

In the current experiment a large proportion of bone demineralization must have taken place to cover the P requirement of lactating sows. As bone ash contains 36% Ca and 17% P (Jongbloed, 1987), it can be estimated that sows receiving the negative control diet an amount of 8.4 g P.kg^{-1}.day^{-1} might have been reabsorbed from bone. Simultaneously, almost 18 g Ca is resorbed from bone. This amount of Ca together with the dietary supply of Ca (32 g) covers almost the daily required amount of Ca (55g). In the phytase-supplemented diets of our experiment the requirement of dP was met for 90%, while for Ca this improved from 59, 75, 85 and 101% respectively, at 125, 250, 500 and 1000 U.kg^{-1} diet, because dietary Ca concentrations were increased.

Kemme et al. (1997a) noted that P digestibility in both unsupplemented and phytase-supplemented diets was lower in breeding sows compared with growing-finishing pigs. Maybe in adult pigs absorptive capacity is reduced resulting in no further improvement of P digestibility.

Another possibility could be a negative effect of bone demineralization on absorption of P, which is also speculated by Lyberg et al. (2007). In most cases the supply of Ca was also suboptimal, which via hormonal regulation (parathyroid hormone), may also have stimulated the mobilization of Ca and P from bone.

The effect of such a high quantity of Ca, which comes into circulation, on Ca and P metabolism is not known. However, it is striking that Ca digestibilty of the diets is much lower than adopted for assessing Ca requirements of sows (45%; Jongbloed et al. 1992). This may indicate that Ca absorption is depressed due to large quantities of Ca that come into circulation from bone demineralization. To get a proper explanation why P digestibility is not further increased at a higher phytase dose, further research is necessary to monitor bone demineralization during lactation and mineral balance studies.

5. Conclusions and implications

Phosphorus and calcium digestibility were clearly enhanced by the addition of microbial phytase to breeding sow diets. In addition, the digestibility of other minerals such as Cu, Zn, Mg, Na and K were improved in lactating sows and to a less extent in gestating sows. Both in gestation and lactation, the maximum effect of the enzyme Optiphos^{TM} was already realized at a dose of 125 U.kg^{-1} diet, without further improvement of efficacy at higher doses. It is interesting to know the relationship between generated dP and doses of Optiphos^{TM} between 0 and 125 U.kg^{-1} of diet in both gestating and lactating sows. In gestating sows the amount of generated dP by microbial phytase was highest at the end of pregnancy, because during mid-pregnancy more dP was fed than required. Available literature showed that the difference in effect in generated dP between mid pregnancy and end of pregnancy was on average 0.47 g.kg^{-1} diet. Therefore, it can be recommended to test efficacy of microbial phytase in pregnant sows only at the end of pregnancy.

Based on this research and available literature, no differences in effect of microbial phytase could be demonstrated between mid and end of lactation. It may be recommended to investigate the reasons why no further enhancement of phytase efficacy can be monitored in lactating sows, even when they are fed below their requirement for dP. No signs of any adverse effect of Optiphos^{TM} phytase were observed on sow or piglet health and performance. No differences between dietary treatments were present in this study.

When an amount of 125 U.kg^{-1} is exchanged for monocalcium phosphate to supply similar amounts of digestible P to the sows a reduction of P excretion can be obtained of 0.85 kg.sow^{-1}. year^{-1}. Thus by using microbial phytase in the sow diets, the reduction in P excretion is beneficial for the environment.

6. References

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